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BIOPOLYMERS VOL. 13, 345–357 (1974)

Circular Dichroism of Films of Polynucleotides

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Synopsis

The CD spectra of films of the lithium salt of *E. coli* and calf thymus DNA, and alternating d-AT:AT were measured as a function of relative humidity. Films of the ammonium acetate salt of DNA were also measured. The ammonium films yield the previously reported A-form CD spectra. A possible explanation for the small magnitude of the 260-nm band of the A-form film spectra compared to double-stranded RNA spectra is that the film DNA is in a different conformation than RNA within the A family of conformations.

At relative humidities of 92% or lower, a negative nonconservative CD spectrum with negative minima near 270 and 210 nm is observed with the lithium films. The magnitude of the minima varies from film to film. In films of DNA the magnitude ranges from a delta epsilon of −5 to −35; d-AT:AT films show magnitudes to −300. CD spectra of this type are designated Ψ spectra. Similar spectra have been reported from reconstituted complexes of DNA and polylysine or f-1 histone. If the origins of the film and protein–DNA complex spectra are similar, the complex spectra are not the result of specific secondary structural changes induced in the DNA by the protein fraction. Theoretical analysis suggests that Ψ spectra are not the result of changes in the secondary or tertiary structure of DNA. Instead, the previously proposed explanation based on liquid crystals is favored. The DNA could form asymmetric structures with long-range periodicity. It is likely that the observed CD spectra of f-1 complexes are artifacts of DNA aggregation. The possibility that some other previously published spectra of protein–DNA complexes also reflect artifacts is suggested.

INTRODUCTION

One approach to studying nucleic acid-containing biological structures is to determine the conformation of the nucleic acid polymer in the structure. Circular dichroism spectroscopy is a potentially useful tool for studying nucleic acid conformation.[1–3] The interpretation of CD data is difficult however, because at present there is no adequate theory that can relate the composition and conformation of a nucleic acid polymer to its measured CD spectrum. In the absence of such a theory, it is necessary to study the CD of polynucleotides under a variety of physical conditions. The CD spectra of biological structures can be compared to other polynucleotide CD spectra, and in this manner the biological data can sometimes be interpreted.

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The present paper reports the CD spectra of films of the lithium salt of *E. coli* DNA, calf thymus DNA, and double-stranded alternating deoxyadenosine and thymidine (d-AT:AT), which have been equilibrated against various relative humidities. The techniques follow those of Tunis-Scheider and Maestre.[4,5] Also reported are the spectra of films of the ammonium salt of *E. coli* DNA. These data should be useful in interpreting many of the results of CD studies of nucleohistone complexes.

MATERIALS AND METHODS

DNA Preparation

E. coli and calf thymus DNA were obtained from Worthington Biochemical Corporation, and d-AT:AT was obtained as a solution in 0.1 *M* Tris buffer from Biopolymers. *E. coli* and calf thymus DNA were dissolved in about 1.5 ml of 10^{-3} or 5×10^{-4} *M* LiCl or 10^{-3} *M* NH_4CH_3COO to form a very concentrated, viscous solution. The resulting DNA solutions were sheared 15 times through a 25-gauge hypodermic needle to reduce the molecular weight. DNA of low molecular weight is easier to make into films and avoids some of the problems with orientational artifacts. The DNA solutions were dialyzed exhaustively against 10^{-3} or 5×10^{-4} *M* LiCl or 10^{-3} *M* NH_4CH_3COO prepared with glass distilled water. d-AT:AT was concentrated by allowing several milliliters of 9 OD/ml d-AT:AT solution inside of a dialysis bag to evaporate to reduced volume. The resulting solution was exhaustively dialyzed against 10^{-3} *M* LiCl.

Preparation of Films

The polymer films were formed on quartz plates 2 cm square. Using disposable micropipets, 50–150 μl of polymer solution were deposited in the center of the plate and spread with a glass rod to cover an area about 1 cm in diameter. The films were stirred with an up-and-down motion of the rod to avoid orienting the polymer in the plane of the plate. We found it easiest to form films when the polymer concentration was about 25 OD/ml. The salt content of the films was determined as previously described.[5] A mean molecular weight of 327 per residue and an extinction coefficient of 6.46×10^3 for *E. coli* DNA were assumed for that determination.

Humidity Chambers

The relative humidity of the films was controlled by placing them inside sealed humidity chambers. The chambers have been described in greater detail elsewhere.[5] The chambers were used in a horizontal position, with the instrument light beam passing through in a vertical direction. The polymer film was deposited on the bottom quartz plate. The relative humidity inside the chambers was controlled by including a saturated solution of an appropriate salt. The salts and their corresponding relative humidities

were Na_2HPO_4, 95%; $NaC_2H_3O_2$, 92%; BaCl, 88%; KBr, 84%; $NaClO_3$, 75%; and $NaNO_2$, 66%. When the relative humidity was changed, films were allowed to equilibrate for at least 24 hr before CD measurements were taken. The chambers, once mounted in the instrument, could be rotated 360 degrees about the optical axis or tilted ±30 degrees.

Modification of Instruments

CD measurements were made with a 6001 CD attachment to a Cary 60 spectropolarimeter. The instrument was modified so that the light beam and 6001 optical unit can be placed on a vertical axis, as well as in the normal horizontal mode. The modification is the same equipment described previously.[5] The instrument light beam is deflected into the vertically mounted optical unit by a front surface mirror placed in front of the Pockel's cell. Absorption measurements were done with a Bausch and Lomb spectronic 600 spectrophotometer. The spectrophotometer was placed in the vertical mode by bolting the instrument on a frame and tilting it 90 degrees on its end.

A special cell holder was used, which can hold a standard 1-cm pathlength cell in both the vertical and horizontal modes. Employing this cell, we checked the calibration of both orientation modes of the Cary 60 at 20°C with a solution of D-10 camphosulfonic acid (1 mg/ml). We also checked both modes for nonactive sample artifacts using a potassium dichromate solution (60 mg/l in 10^{-2} M H_2SO_4) from 540–190 nm. The maximum artifact was less than 1 millidegree/OD of nonactive sample.

Measurement of Films

Absorption spectra of the films were recorded from 350–220 nm before every CD measurement. The cells were positioned in the spectrophotometer so that the light beam impinged on approximately the same part of the film as in the Cary 60. The amount of polymer in the light path was determined from the absorption at 260 nm and the extinction coefficient of the polymer in solution. The absorption reading at 350 nm was subtracted from the 260-nm reading for this determination. DNA has no absorption at 350 nm. Any extinction in this region is due either to reflection from the various surfaces or to light scattering from the films, which is sometimes apparent at low humidities. The absorption baseline for an empty humidity chamber is flat. If scattering contributes to the 350-nm extinction however, there will be some error in the above procedure for determining absorption, since scattering extinction varies with wavelength. The extinction at 350 nm amounts to about 10% or 20% of the extinction at 260 nm, when the spectrophotometer is adjusted to read zero extinction at 350 nm with no chamber in the light path.

The CD baseline of the empty humidity chamber complete with salt boat and windows was recorded before each film was formed. The baselines of the three chambers used in these experiments are reproducible,

similar to each other, and relatively flat down to 220 nm. At 220 nm the baselines curve significantly and are not always reproducible. There may be errors in the reported CD spectra below 220 nm due to distorted baselines. These errors are small compared to the large ellipticities we observe in the lithium polynucleotide films, but they could be significant in the ammonium DNA spectra (see below). Since a film may be studied for periods of up to a week or more, the CD machine baseline may change in that time. When a film is measured several days after it is formed, a new baseline is run using another empty cell. All scans are made from 350 nm, and the pen position is adjusted to give a zero reading in that region. Circular dichroism is reported in units of $\epsilon_L - \epsilon_R$, where ϵ_L is the per residue extinction coefficient of left circularly polarized light in l/mol·cm.

RESULTS AND DISCUSSION

Ammonium DNA Films and A-Form Spectra

In order to provide a check on our present technique of making films as well as on past results[4,5] we measured the CD spectra of films of the ammonium acetate salt of *E. coli* DNA. Figure 1 shows the spectra of such a film at several relative humidities. The peak at 270 nm in the wet film shifts about 10 nm toward the blue and a negative minimum at 210 nm develops as the relative humidity is lowered. At low relative humidities a negative long wavelength band appears in the spectrum at around 290 nm. These results are in agreement with previous work[4,5] and have been interpreted as representing the CD spectrum of DNA in the A-form conformation.[6] In some films the initial negative minimum is quite large and the spectra are nearly conservative in the 240–300-nm region. This conservative spectrum is illustrated in Figure 2. The sign of the conservative spectral component is opposite to the sign of the conservative component of the B-form CD spectrum.

The most striking feature of the ammonium salt film spectra is the large magnitude of the 210-nm minimum relative to the 235–245-nm minimum. The increase in magnitude of the 210-nm minimum relative to the 235–245-nm minimum as the film goes into the A form also occurs in the previously reported spectra of the films of the sodium and ammonium salt of *E. coli* and calf thymus DNA,[4,5] and is probably the most characteristic feature of A-form DNA CD spectra, at least for these nucleic acids.

The A-form film DNA CD spectra have all the features of double-stranded RNA CD spectra,[7] except that the magnitudes of the positive 260-nm maximum are too small. The 260-nm maximum of double-stranded RNA of rice dwarf virus has a magnitude of about $\Delta\epsilon = 7$; our films have magnitudes for that maximum of about $\Delta\epsilon = 3$–4. Recently Ivanov et al.[8] measured the CD spectrum of calf thymus DNA in 78% ethanol and found it to have features of an A-form spectrum, including a magnitude of the 260-nm maximum of about $\Delta\epsilon = 9$ and a large negative 210-nm minimum.

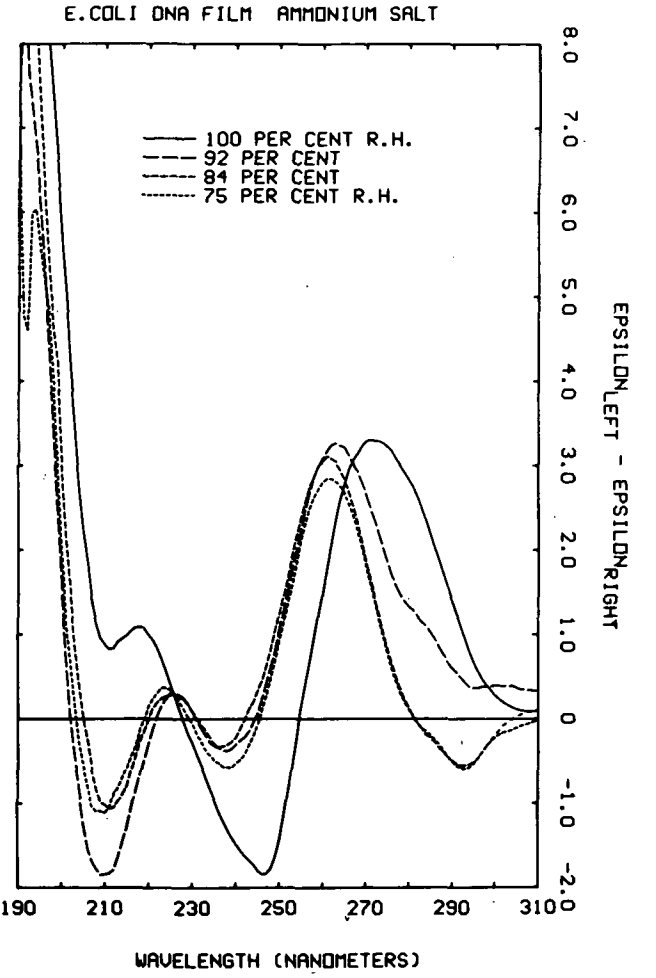

Fig. 1. Film of ammonium salt of *E. coli* DNA as function of relative humidity.

There are two possible explanations for the small magnitude of the 260-nm peaks of the film spectra. First, the transformation of DNA to the A form could be incomplete, leaving part of the DNA in another conformation. Alternatively, the DNA in the films could have a conformation that is in the family of A-form conformations, but that is somewhat different from that of double-stranded RNA of rice dwarf virus or DNA in 78% ethanol. Ivanov et al.[8] describe the various DNA conformations as belonging to two families: the B type and the A type. The two families are differentiated by the pucker of the ribose ring, which is C3-exo for the B family and C3-endo for the A family,[9] as well as by other well-known parameters such as base twist and tilt. Within families, various conformations are generated by changes in the winding angle and small changes in twist and tilt. Thus, B- and C-form DNA are within the B family, and A and

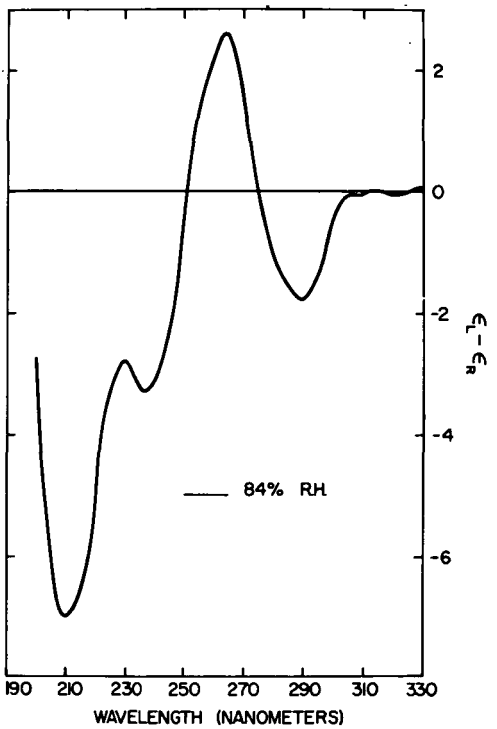

Fig. 2. Film of ammonium salt of *E. coli* DNA at 84% relative humidity showing conservative A-form CD spectrum.

A′ RNA are within the A family. Changes of polymer conformation within a family are influenced by changes in the salt in the polymer environment.[8,10] It is conceivable that our A-form film CD spectra represent the spectrum of an A-family DNA conformation, which is produced by the particular salt conditions of the films. Such a result would be analogous to the CD changes in DNA as it goes from the B conformation in dilute salt to the C conformation in high salt solutions. In the C conformation the magnitude of the long wavelength peak is much less than for the B conformation, while the short wavelength region of the CD spectrum is nearly unchanged.[4] It appears probable that there is not just one A-form DNA CD spectrum, but rather a whole family of them. There is the nonconservative spectrum seen in DNA in 78% ethanol, with a large 265-nm positive maximum and a weaker negative minimum at 245 nm. In double-stranded RNA of rice dwarf virus a negative band appears in the spectrum at 295 nm, and in some DNA film spectra that negative band is nearly as strong as the 260-nm positive peak, giving a conservative long-wavelength spectrum as in Figure 2.

Lithium Films and Ψ-Form Spectra

As wet films (100% relative humidity) of the lithium salt of DNA are brought to relative humidities of about 92% or less, a dramatic change

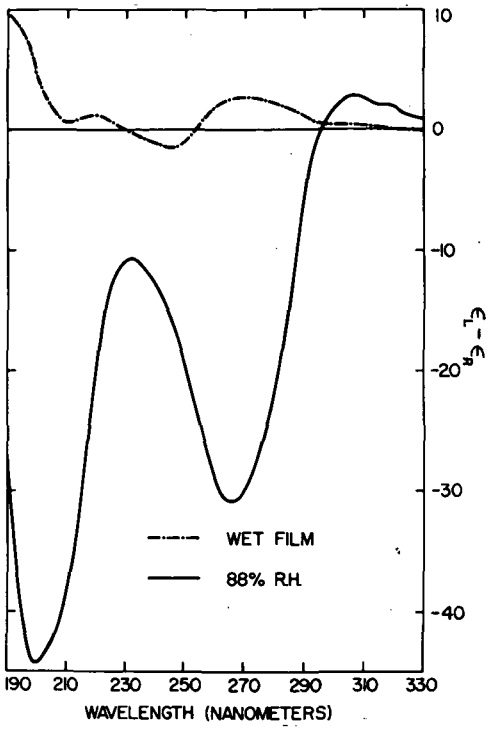

Fig. 3. Film of lithium salt of *E. coli* DNA at 100% and 85% relative humidity.

occurs in the CD spectrum. Figure 3 shows the CD spectra of a wet film of *E. coli* DNA, and of the same film equilibrated against 88% relative humidity (RH). The 100% RH CD spectrum resembles the solution spectrum for *E. coli* DNA. The 88% spectrum is nonconservative over the observable spectral range, with a large negative minimum at 265 nm, and another, larger negative minimum at 202 nm. The small positive peak at 305 nm is probably an artifact due to differential light scattering, since DNA does not absorb light in that region. This particular film, however, did not show the increased extinction at 350 nm upon dehydration to 88% RH, which implies light scattering by the film. Once the relative humidity is lowered to about 92%, further change in relative humidity does not influence the CD spectrum significantly. Figure 4 shows the CD spectra of one film studied at several relative humidities. The lithium salt concentration of both these films was between 2% and 4%.

Figure 5 shows the CD spectrum of a film of the lithium salt of d-AT:AT at 95% RH. The spectrum is similar in shape to the low-humidity spectra of the DNA films, but the magnitude of the d-AT:AT CD minima is much greater than for DNA. The CD minima in Figure 5 are at 270 and 210 nm.

To investigate the influence of salt concentration, we prepared *E. coli* DNA films with 0.5%, 1%, and 2% LiCl by weight. In each case negative

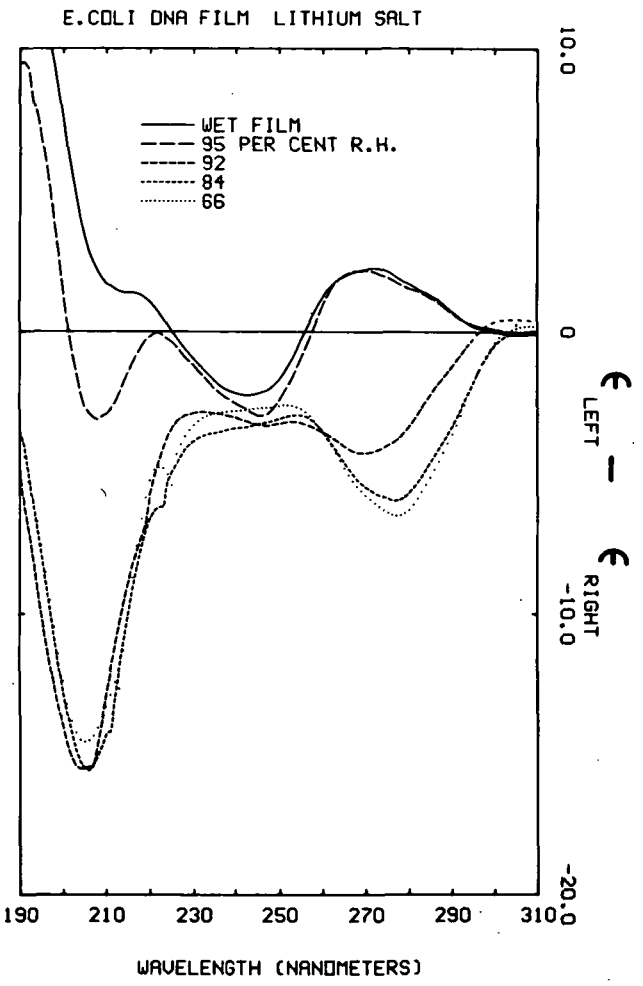

Fig. 4. Film of lithium salt of *E. coli* DNA as function of relative humidity.

nonconservative CD spectra with minima at about 265 and 205 nm were observed when the films were dehydrated to 92% RH. Similar results were obtained with films of calf thymus DNA.

The spectra in Figures 3–5 are typical of the film spectra we have obtained with the lithium salt of DNA or d-AT:AT. These results can be summarized by describing the spectra as negative and nonconservative, with a negative minimum between 265 and 270 nm, and another greater negative minimum between 200 and 210 nm. The magnitude of these nimima is not a distinctive feature of these spectra; it varies from film to film and must be influenced by the conditions of film preparation. The range of magnitudes of these minima is from -5 to -300. Films of d-AT:AT always give much larger minima than films of DNA. Similar spectra have been reported from solutions of DNA with high concentrations of neutral

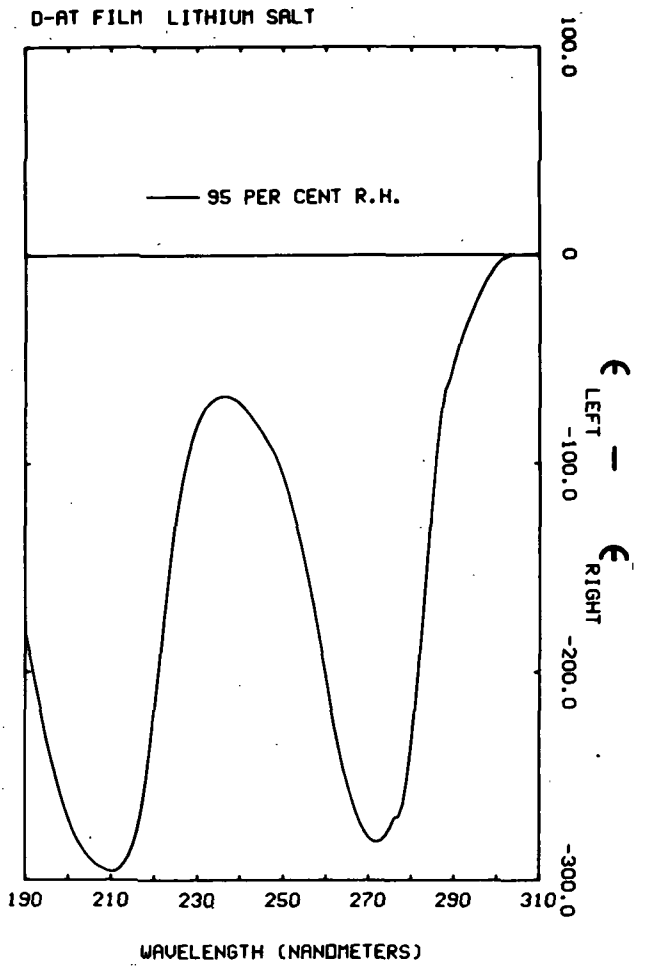

Fig. 5. Film of lithium salt of d-AT:AT at 95% relative humidity.

polymer.[11] Following the terminology of Jordon et al.[11] we will designate the above described spectral form as a Ψ-type CD spectrum.

To examine the possibility that the Ψ-form spectrum is an artifact resulting from orientation of the polymer on the quartz plates, several films were measured again after the chambers were rotated or tilted. The shape and magnitude of the film CD spectra were independent of rotation about the optical axis. The shape of the spectra also remained constant when the chambers were tilted, although sometimes the magnitude of the minima changed. The change of magnitude upon tilting can be explained by the fact that the light beam of the instrument passes through a different part of the film when the humidity chamber is tilted, and the films are not entirely uniform. We conclude that the Ψ spectrum is not an artifact due to orientation of the sample.

CD spectra with the same features that we have described for Ψ-form film spectra have been observed by Tunis-Scheider and Maestre[4] in some films of the ammonium acetate salt of calf thymus DNA. Ψ-type CD spectra have also been observed for a variety of reconstituted nucleohistone complexes and model compounds. ORD Cotton effects consistent with Ψ-type spectra have been reported in DNA complexed with poly-L-lysine.[12,13] Ψ-type CD spectra of DNA–polylysine complexes have also been observed by several authors.[14,15] The magnitude of the CD minima or Cotton effects is not constant; it varies from preparation to preparation.[13] Ψ spectra have also been reported from complexes of the lysine-rich histone fraction f-1 with DNA.[15–19] Again the magnitude of the minima varies from preparation to preparation, but most of the spectra exhibit minima with magnitudes between −5 and −35. The origin of the Ψ-type CD spectra in protein–DNA complexes has been attributed to changes in the secondary structure of the DNA molecule as it complexes with the protein fraction,[12,13] or to changes in the DNA secondary structure specifically induced by the protein component as the complexes aggregate into larger particles.[16–19]

It is reasonable to assume that the Ψ-form film and protein complex spectra have a similar origin. In that case the Ψ spectrum cannot be due to changes in DNA specifically induced by any protein. It is conceivable that the Ψ-type spectrum arises from interactions between bases of DNA that is in some new double-stranded conformation, much as the A- and B-form CD spectra are assumed to be produced.[20] The most prominent feature of the Ψ-type spectra is that they are overwhelmingly nonconservative throughout the entire observable spectral region. Theoretical treatment of polymer CD demonstrates that CD spectra that are nonconservative in a given spectral region must be due to interactions with transitions outside that region.[21] If Ψ spectra did reflect secondary DNA conformational changes, the CD spectra would be due to very strong interactions between the 260- and 210-nm absorption bands of DNA and the far uv transitions beyond 185 nm.

Neither the A- nor the B-form DNA CD spectra are nearly as nonconservative as the Ψ spectrum. Recent calculations have shown that it is possible to explain the A- and B-form spectra in a qualitative, approximate manner by interactions among nucleic acid base transitions in the near uv region.[22] If the Ψ-type spectrum repesents some new conformation of DNA, there would have to be much greater interaction between the near and far uv transitions in the Ψ form than in either the A or B geometries. That fact suggests that the hypothetical Ψ geometry would be considerably different from both the A and B structures.

There have been a variety of X-ray scattering studies on nucleohistone in gels and solution.[14,23–25] Although the resolution is poor, all the studies agree that the conformation of DNA in nucleohistone is very near to the B geometry. Some X-ray studies have suggested that the DNA might be supercoiled,[24,25] but measurements have shown that the CD spectrum of supercoiled DNA is very similar to that of the B form.[26] It is unlikely that

the Ψ-type CD spectrum is produced primarily by any change in the secondary or tertiary conformation of the DNA molecule. Interpretation of Ψ-form CD spectra from DNA protein complexes in terms of changed local DNA structure is probably erroneous.

The recent X-ray work of Suwalsky and Traub[27] does not deal with the structure of Ψ-form DNA since they made their DNA–protein complexes by direct addition, rather than by annealing through salt gradient dialysis. Previous CD studies indicate that DNA in direct addition complexes is in the B form.[15]

Gottesfeld et al.[28] have proposed that the Ψ-type spectrum might be an artifact resulting from differential light scattering from reconstituted nucleohistone complexes. Recently Dorman and Maestre have developed techniques to estimate the contribution of differential light scattering to measured CD.[29,30] Those techniques have been applied to complexes of DNA with f-1 histone.[31] The results demonstrate that while there is a contribution to f-1 DNA CD spectra due to differential light scattering, it is relatively small and cannot account for the observed features of the Ψ-type CD spectrum. Šponar and Frič[32] suggested on the basis of their observed dependence of the magnitude of the Ψ-type spectrum on the G–C content of DNA that the Ψ spectrum is due to a conformational change of the G and C bases and an interchain interaction between G bases. We have observed the strongest Ψ-type spectra in films of d-AT:AT however, so the role of guanine–guanine interactions in the production of Ψ-type CD spectra cannot be decisive.

Several authors[13,14] have proposed that the Ψ-type spectra of nucleohistone complexes results from liquid crystals.[33] If DNA molecules aggregate to form large, periodic, asymmetric structures, very large optical rotations could be expected. Tunis-Schneider and Maestre[4] suggested that DNA aggregation into quasicrystalline microdomains was responsible for the large CD values they observed in some of their films. Clearly the Ψ spectra result from some kind of aggregation; such spectra are found when DNA is highly concentrated in films or brought together in histone complexes. The appearance of Ψ spectra in f-1 histone complexes is dependent upon aggregation of the complexes.[14,16,19] Histone-complex spectra are also concentration dependent. Intense Ψ spectra are observed at lower histone to DNA ratios in 10^{-3} *M* DNA than in 10^{-4} *M* DNA.[16] Holzwarth and Holzwarth[34] have shown that liquid crystals can produce strong nonconservative CD spectra within the absorption bands of the crystal. Calculations by Holzwarth et al.[35] further indicate that long-range periodic asymmetric structure contributes to the observed CD spectrum of T2 phage. The liquid crystal theory also provides an explanation for the fact that the magnitudes, but not the shapes, of the Ψ spectra vary over a wide range. We believe the liquid crystal approach provides the most likely interpretation of these spectra.

The conditions under which our lithium polynucleotide films were prepared have been previously reported[4] to cause films to exhibit a different kind of CD spectrum than the one we report here. The previously observed

spectrum was identified with the C-form geometry of DNA.[36] The conditions under which lithium films exhibit the C-form spectrum, and the conditions under which the DNA aggregates to yield a Ψ-form spectrum, are presently not understood. However, it is known from X-ray fiber work that the salt content of the fiber as well as the relative humidity is critical to obtaining a C-form X-ray pattern.[36,37] Too much salt as well as too little can prevent fibers from assuming the C form. The salt content of our films has been only crudely estimated. It is possible that a careful study of films of various salt contents would define the conditions for the C- and Ψ-form CD spectra.

CONCLUSIONS

The features we observed in the Ψ CD spectra of films of the lithium salt of DNA and d-AT:AT are similar to the features of some reported CD spectra of DNA–polylysine complexes and complexes of DNA with the f-1 fraction of calf thymus histone. It is reasonable to assume that the origin of these spectra is similar in both the films and the DNA–protein complexes. In that case the CD spectra observed in f-1 complexes are not the result of DNA conformational changes specifically induced by the protein fraction, as has been suggested by Fasman et al.[16–19] It is also unlikely that the Ψ spectra are the result of any DNA conformational change. Probably such spectra arise from long-range periodic asymmetric structures developing as DNA aggregates to form liquid crystals. The CD spectra of f-1 complexes are essentially an artifact and do not reveal information about the secondary structure of nucleohistone. Any CD changes that might occur as a result of DNA conformational changes as the polynucleotide complexes with the protein are masked by the much more intense Ψ spectrum.

CD spectra different from both DNA in solution and Ψ-type spectra have been reported for other protein–DNA complexes.[14,19,28,38–40] Whether these spectra represent specific conformational chnges in DNA, or whether they also represent CD artifacts, is presently unknown. It is evident however, that when unusually large or nonconservative CD spectra are observed from condensed or aggregated samples, one should suspect the possibility of artifacts due to liquid crystal phenomena.

This work was supported by NASA Grant NGR-05-003-460 and USPHS Grant AI-08427-Q4.

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Received September 5, 1973
Revised November 12, 1973